Title of Research : **Structural Investigation of UV-cured DNA Films**

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1.Samples of DNA derived from Salmon roe to send Dr. James G. Grote of US-AFRL

Purity: 95%

Protein content:1.24%

Molecular weight: over 10 Million

2. Research Objectives and Plan by using the above DNA samples

Pure DNA derived from Salmon roe can be fabricated to an uniform films by casing an DNA aqueous solution on to a Tefron-coated glass plate, followed by evaporating water and the thickness of the DNA films can be adjusted from 5~50 micron by changing the concentration of DNA in water and also by casting conditions such as evaporation times and temperatures.

However, DNA molecules are soluble only in water and no other any organic solvents are available. It has been known that DNA molecules can be crosslinked under certain conditions of ultra-violet (UV) light irradiation, so that the UV-irradiated DNA films or fiber become insoluble in water.

It has been reported that the crosslinked structure of DNA molecules might be ascribed to a dimerization of thymin withinin a double helix of DNA molecules, as follows:

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14. ABSTRACT

It is important to establish an insolubilization process of DNA molecules to clarify the UV crosslinking reactions for DNA device applications. Photocrosslinking reactions of DNA films under UV irradiation were investigated in terms of effective UV photo-crosslinking reaction conditions. Various UV-initiators were used in order to prepare water-insoluble DNA films and also UV-curing conditions with or without UV initiators were investigated by using various powerful UV lamps.

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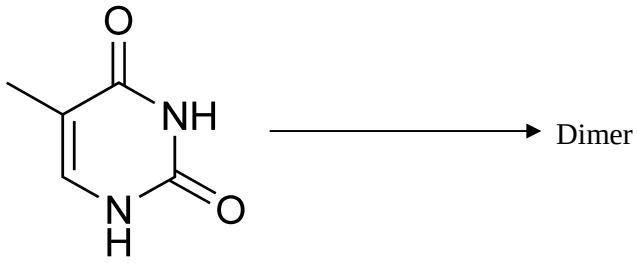


Thymin

However, thymin molecules are fixed in double helical structures of DNA molecules and it would be unreasonable to assume an another thymin molecule is excited to cause a dimerization with an another thymin molecules of another DNA molecules. No clear reports on the crosslinked structures of DNA are available. It is very important to insolubilize DNA molecules in water in terms of DNA photonic or electronic devices. Thus, this investigation to establish an insolubilization process of DNA molecules is very important in terms of device applications of DNA to clarify the UV crosslinking reactions. This research aims at investigations of following UV-curing reactions

3. Research Results

3- 1 Novel crosslinking reaction conditions of DNA films by UV irradiation

Photocrosslinking reactions of DNA films under UV irradiation was investigated in terms of effective UV photo-crosslinking reaction conditions. Various UV-initiators was used in order to prepare water-insoluble DNA films and also UV-curing conditions with or without UV initiators was investigated by using various powerful UV lamps.

Table 1 summarizes results of the UV-crosslinking of DNA films. UV-curing initiators which produced radicals by UV irradiation were effective for the crosslinking reaction of the DNA films in such a short time of 30 sec. by using a normal 1 W UV lamp. However, when a powerful UV lamp made by Moritex Inc, in Japan with an intensity of 160 mW/cm^2 was used for the crosslinking reactions of the DNA films, no UV curing agents were necessary for the crosslinking reactions of the DNA films and water-insoluble and clear transparent DNA films were easily obtained.

Table 1 UV curing conditions of DNA films

UV inititators	Irradiation time	Insoluble
Dalocure Ilugacure	30sec、 1,3,5,10,20min.	○
None	1,2,3,5,10,15,30min.	○

※ UV lamp made by Moritex Inc. UV intencity:160mW/cm2 ）。
※ 10μm DNA-Na films were used。

3-2. Structural analyses of UV crosslinked DNA films

Structures of UV-crosslinked DNA films was investigated by using various infrared spectroscopic methods and also mechanical properties of the crosslinked DNA films was measured in terms of stress-strain curves. Surface structures of the UV-crosslinked DNA films will be investigated by electron microscope or AFM.

Results of the mechanical properties of the crosslinked DNA films made by Nippon Chemical Feeding Company and the Daiwa Chemicals Company were compared in Fig. 2, which indicates that the Daiwa-made DNA film was much stronger than the Nippon Chemical Feeding-made DNA film. Presumably, the difference in mechanical properties might be related with the molecular weight differences between the Daiwa-made DNA and the Japan Chemical Feeding-made DNA, as shown in Fig. 3 as results of electrophreses of the DNA aqueous solutions.

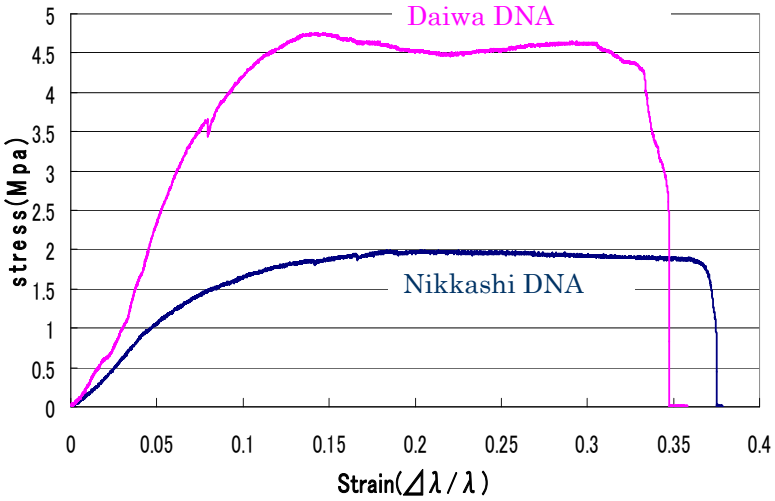


Fig. 2 Stress-strain curves of the crosslinked DNA films

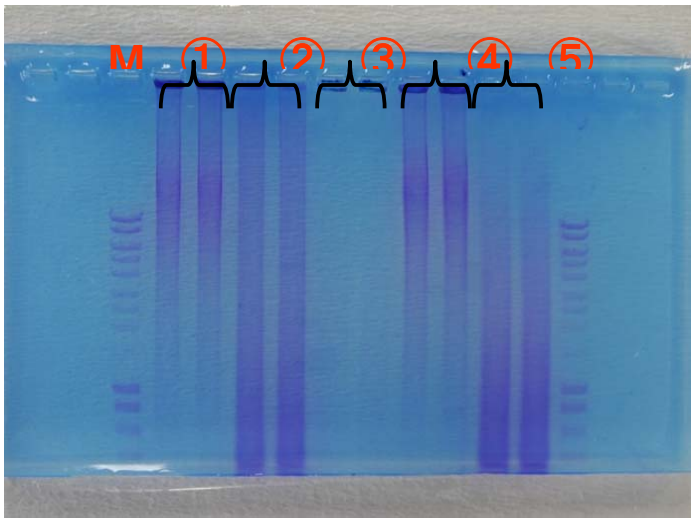


Fig. 3 Electrophoreses of aqueous solutions of various DNA

Sample number

① Japan Chemical Feeding Co.:DNA-Na

② Low molecular weight DNA-Na（Daiwa Chemicals Lot.HP-JG0912、

③ High molecular weight HP-DNA-Na（Daiwa Chemicalks

Lot.HP-JH1004）

④ DNA-Na（Japan Chemical Feeding Co. 2/21）

⑤ Low molecular weight DNA-Na（Daiwa Chemicals Co.、Lot.HP-JG0912、

Infrared spectra of the UV-cured DNA films were measured as shown in Figs.4-6 where IR spectra before and after UV-irradiations were measured as differential absorption spectra in Fig.6, which indicates strong absorption owing to P-O-P bond

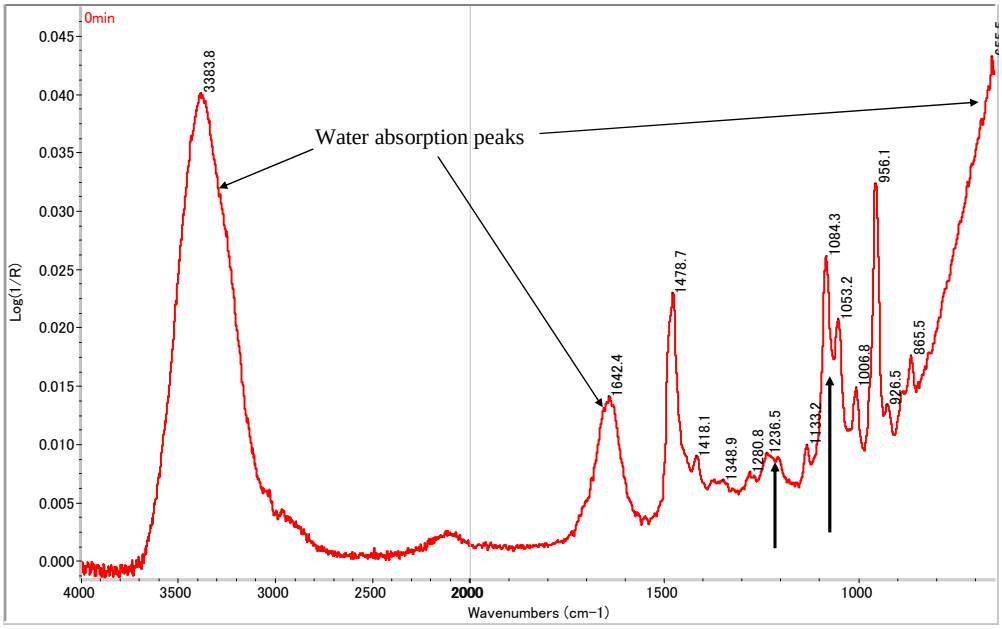


Fig. 4　Infraed spectrum of the DNA film before UV irradiation

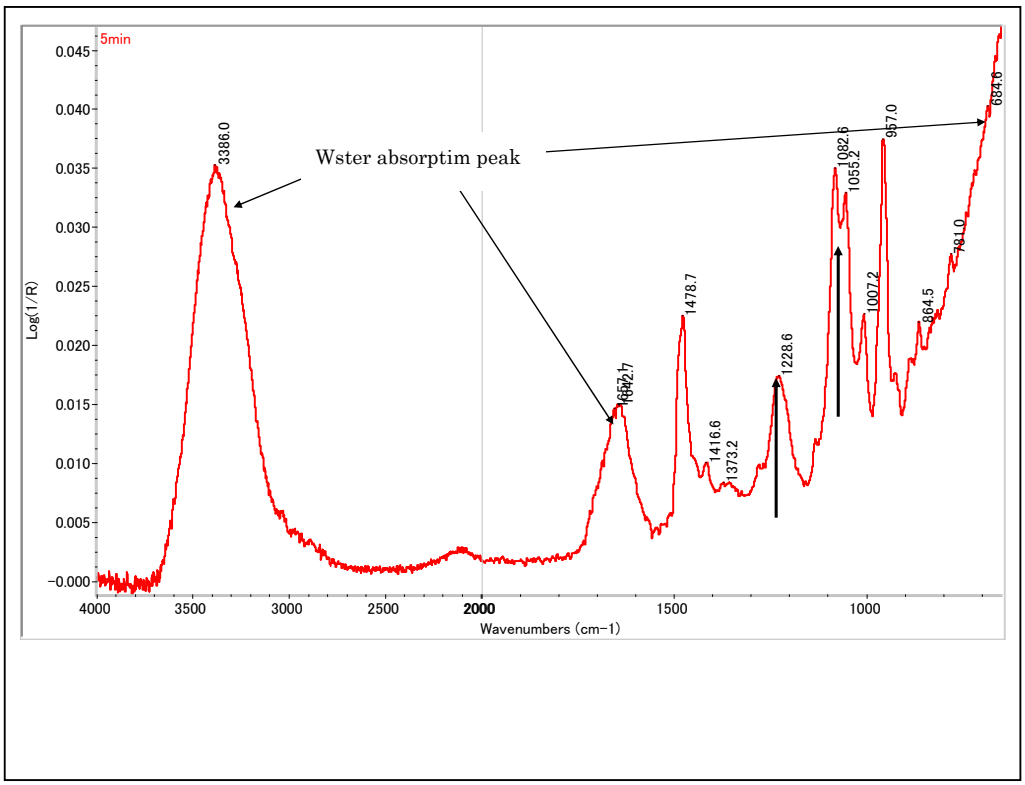
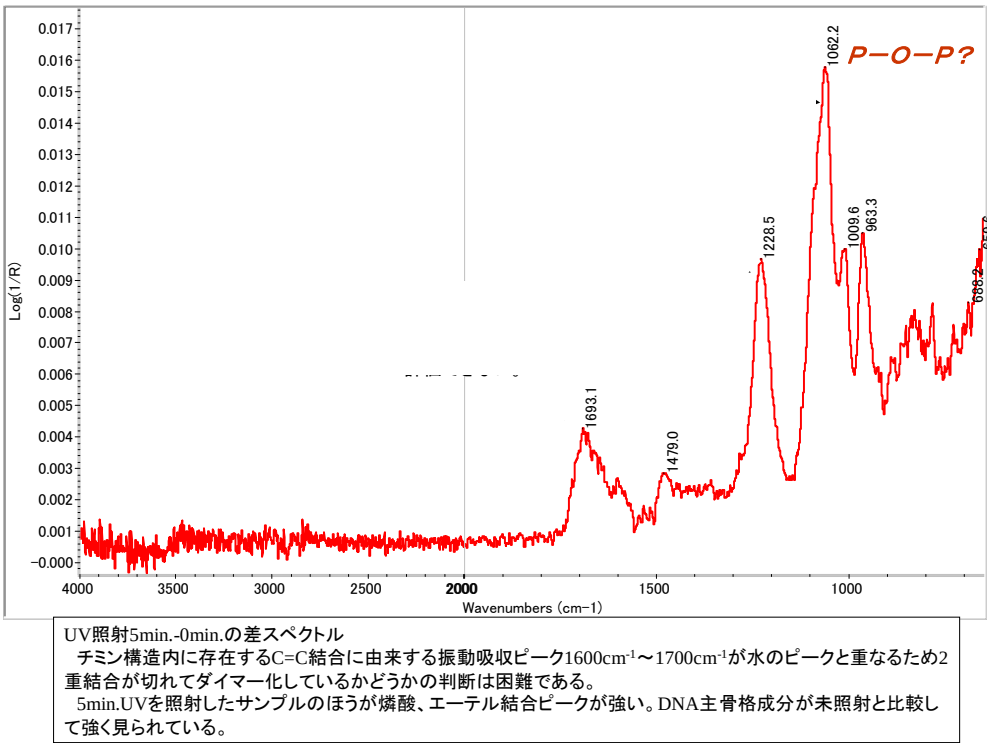


Fig. 5 Infrared spectrum of the UV-crosslinked DNA film after irradiation of 5 min.



UV照射5min.-0min.の差スペクトル
チミン構造内に存在するC=C結合に由来する振動吸収ピーク1600cm^{-1}～1700cm^{-1}が水のピークと重なるため2重結合が切れてダイマー化しているかどうかの判断は困難である。
5min.UVを照射したサンプルのほうが燐酸、エーテル結合ピークが強い。DNA主骨格成分が未照射と比較して強く見られている。

Fig. 6 Differential IR spectrum before and after UV irradiation of the DNA film

The differential IR spectrum strongly suggests the formation of P-O-P bond in the UV-cured DNA film, which caused water-insoluble UV-crossliking reactions among DNA molecules.

Figs 7 and 8 show surface pictures of UV-irradiated DNA films with different thickness as 2,5,7 and 10 μ.. It is seen in Figs.7 and 8 that the UV-crosslinked film with the thickness of 7μ showed a very roughed surfcase, while the thinner DNA film of 2μ showed a fine patterned surface structure. These surface structure difference might be resulted from the crosslinking density of the UV-irradiated DNA films Further precise analyses of the crosslinking density would be necessary.

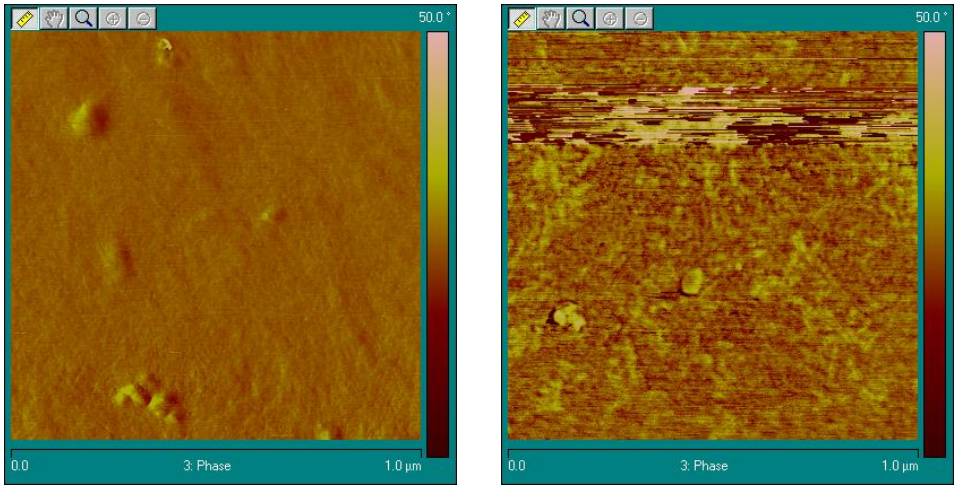


Fig. 7 Surface pictures of 10 and 7 μ films after UV irradiation

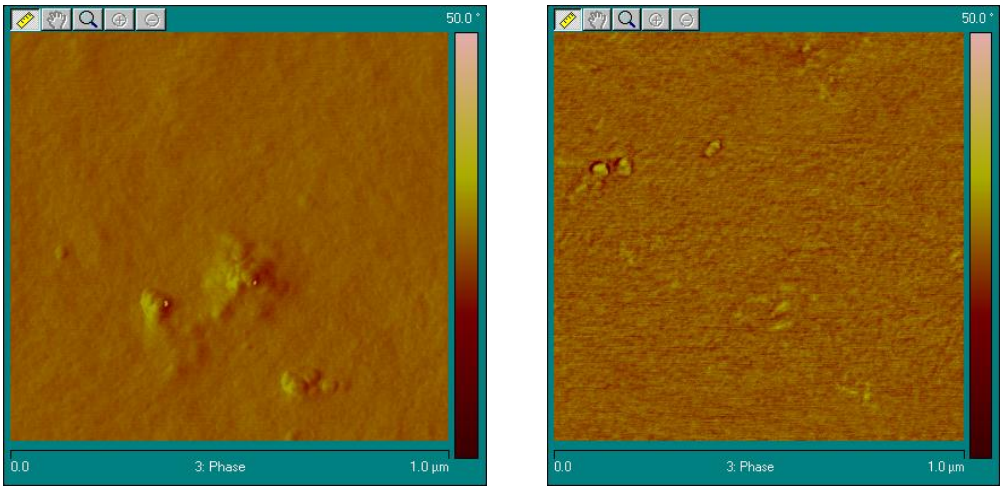


Fig. 8 Surface pictures of 5 and 2μ films after UV irrdiation

4. Summary

when a powerful UV lamp made by Moritex Inc, in Japan with an intensity of 160 mW/cm^2 was used for the crosslinking reactions of the DNA films, no UV curing agents were necessary for the crosslinking reactions of the DNA films and water-insoluble and clear transparent DNA films were easily obtained.

The differential IR spectrum strongly suggests the formation of P-O-P bond in the UV-cured DNA film, which caused water-insoluble UV-crossliking reactions among DNA molecules.

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